Gut 1995; **37**: 524–529

Loss of CD4 T lymphocytes in patients infected with human immunodeficiency virus type 1 is more pronounced in the duodenal mucosa than in the peripheral blood

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Abstract

Although changes in T lymphocyte subset distribution in the peripheral blood of patients infected with human immunodeficiency virus (HIV) are well defined it is not known whether these changes reflect changes in lymphoid compartments clearly involved in HIV related disease like the intestinal mucosa. This study analysed lymphocytes isolated simultaneously from the peripheral blood and duodenal biopsy specimens by three colour flow cytometry in eight asymptomatic HIV infected patients, 26 AIDS patients, and 23 controls. The proportion of CD4, CD8, $CD4^-CD8^-$, or γδ T cells did not correlate between circulating and duodenal T cells. CD4 T cells were reduced in the peripheral blood (7·5% (25th-75th percentile, 2–16%) *v* 52% (41–63%), $p<0·0005$) and even more reduced in the duodenum (1% (1–2%) *v* 36% (23–57%), $p<0·0005$) of AIDS patients compared with controls. Patients with asymptomatic HIV infection had intermediate CD4 T cells in the peripheral blood (24% (22–35%); $p<0·002$ *v* controls; $p<0·01$ *v* AIDS) but like AIDS patients very low CD4 T cells in the duodenum (3% (1–6%); $p<0·002$ *v* controls). The ratio of duodenal to circulating $CD4^+$ T cells was significantly reduced to 0·2 (0–1) in AIDS patients ($p<0·001$) and even to 0·1 (0·04–0·5) in asymptomatic HIV infected patients ($p<0·002$) compared with 0·72 (0·44–0·95) in controls. These findings show an early and preferential loss of duodenal CD4 T cells in HIV infection. Immunological abnormalities in HIV infection are distinct between lymphoid compartments, and profound immunodeficiency may occur in the intestinal immune system although circulating T cells are largely preserved.

(*Gut* 1995; **37**: 524–529)

Keywords: small intestine, mucosal immunology, flow cytometry, HIV, AIDS, macrophages.

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Accepted for publication 24 February 1995

The gastrointestinal tract is a common site of manifestations defining AIDS and is thus obviously involved in the pathogenesis of human immunodeficiency virus (HIV) infection. Effects of HIV infection, however, on the gut mucosal immune system, which comprises the largest amount of immune cells in the body, are not well defined.[1] Although changes in T lymphocyte subset distribution in the peripheral blood of patients infected with HIV have been studied extensively,[2 3] it is doubtful whether these abnormalities reflect changes in other lymphoid compartments[4] for several reasons. Firstly, recent data suggest that the HIV burden may be significantly higher in the intestine[5] or other lymphoid tissue[6–8] compared with peripheral blood. Secondly, it has been shown that HIV replication and cytopathicity is strongly affected by the state of activation[9] and differentiation[10 11] of T cells, which clearly differs between mucosal and circulating lymphocytes.[12 13] There are only few immunohistological studies describing a variable decrease in intestinal CD4 lymphocytes in the mucosa of HIV infected patients,[14–19] and comparative analyses of circulating and intestinal T cells in HIV infection are missing. Therefore we studied lymphocytes isolated at the same time from the peripheral blood and the duodenal mucosa by three colour flow cytometry. Our findings show that the loss of $CD4^+$ T lymphocytes in HIV infected patients is more pronounced in the duodenal mucosa than in the peripheral blood.

Methods

Patients

We studied 34 HIV infected patients who had diagnostic upper endoscopy because of gastrointestinal symptoms. According to the definition of the Centers for Disease Control[20 21] eight patients had asymptomatic HIV infection, the other patients had AIDS. The symptoms leading to endoscopy of our patients were diarrhoea in six, dysphagia in eight, weight loss in seven, nausea in seven, abdominal pain in five, and epigastric pain in one patient. Infectious agents were detected in duodenal biopsy specimens of six patients (Microsporidia and *Giardia lamblia* each in two, cytomegalovirus and *Mycobacterium avium* complex each in one patient). All patients were white, 31 were homosexual or bisexual men, three were injecting drug users (two men, one woman). The patients ranged in age from 28 to 65 years (median 38). Twenty eight patients received zidovudine treatment at the time of study. The control group comprised 23 patients (14 men and nine women;

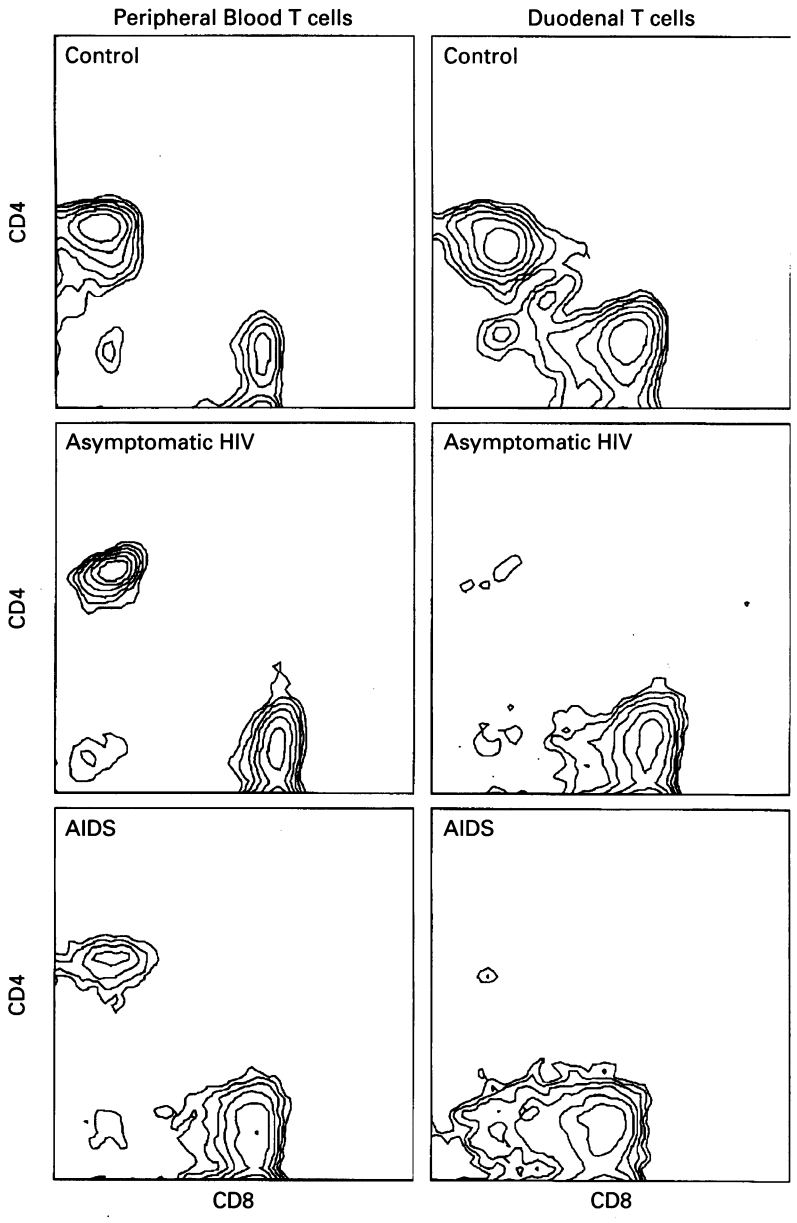

Figure 1: Contour blots of two colour immunofluorescence profile of gated CD3+ T cells isolated from the peripheral blood and duodenum of a control patient (77% and 35% CD4+ T cells, respectively), of an asymptomatic HIV infected patient (3% and 4% CD4+ T cells, respectively), and an AIDS patient (16% and 1% CD4+ T cells, respectively). Logarithmic intensities for red fluorescence (CD4) and green fluorescence (CD8) are shown on the y axis and x axis, respectively. The lines on the graph represent a 'contour' map connecting equal proportions of the total cell population.

aged 26–68, median, 39 years) not at risk for HIV infection undergoing diagnostic upper endoscopy because of epigastric pain (n=19) or weight loss (n=4) who had no abnormalities at histological examination of duodenal biopsy specimens. The study was approved by the local ethics committee.

Investigations

From all patients and controls duodenal biopsy specimens (n=5–9) and venous blood were obtained at the same time. Samples were taken from macroscopically normal areas of the distal duodenum.

Isolation of lymphocytes

Peripheral blood lymphocytes were isolated by Ficoll (Pharmacia, Uppsala, Sweden) density gradient centrifugation. Intestinal lymphocytes were isolated from duodenal biopsy specimens by a modified method used for the isolation of mucosal lymphocytes from resected specimens previously described.[22] Samples were washed in phosphate buffered saline (PBS) and cut into small pieces. After a second washing step the fragments were incubated overnight at 4°C in RPMI 1640 medium containing 10% fetal calf serum (Gibco BRL, Berlin, Germany), 25 mM HEPES buffer, 0·05 M 2-mercaptoethanol, 100 U/ml penicillin, 100 µl/ml streptomycin, 50 µg/ml gentamycin, 2·5 µg/ml amphotericin (Biochrom KG, Berlin, Germany), 0·01% collagenase CLS III (Worthington Diagnostic Systems, Freehold, NJ, USA), 0·01% desoxyribonclease I (Boehringer, Mannheim, Germany), and 0·01% soybean trypsin inhibitor (Sigma, Deisenhofen, Germany). Afterwards the medium with the mucosal fragments was incubated on a shaker for three hours at 37°C. For further disintegration the suspension was passed first for several times through a spinal needle and then through a 60 µm nylon net. The resulting single cells were washed, resuspended in 30% isotonic Percoll solution (Pharmacia, Uppsala, Sweden), and underlayered with 70% isotonic Percoll. After centrifugation intestinal lymphocytes consisting of lamina propria lymphocytes and intraepithelial lymphocytes were obtained from the interface and washed with RPMI 1640 containing 10% fetal calf serum. The number of mononuclear cells obtained was $0{\cdot}9–2{\cdot}9\times10^6$, and viability as determined by trypan blue dye exclusion was greater than 75%.

Immunofluorescence studies

Isolated peripheral blood lymphocytes and intestinal lymphocytes were stained for three colour immunophenotyping by flow cytometry. Immediately after isolation cells were fixed in 1% paraformaldehyde to reduce infectivity, to prevent receptor internalisation, and to stain only cell surface molecules. The following directly fluorescein-isothiocyanate-, phycoerythrin- or peridin chlorophyll protein reagent conjugated monoclonal antibodies were used: anti-CD8 and anti-CD4 from Dakopatts, Glostrup, Denmark, anti-CD3 and isotype matched control antibodies from Becton-Dickinson, Heidelberg, Germany, anti-γδ T cell receptor from T cell Science, Cambridge, USA. Staining was performed in 96 well round bottomed microtitre plates. Previous experiments showed no significant differences in the percentage of positive cells between fixed and unfixed cells and between one step and successive staining for the antibodies used in this study. Thus, for each sample all three antibodies were incubated in one step for 30 minutes at 4°C in the dark with 100 µl staining buffer (PBS containing 2% fetal calf serum and 0·01% sodium azide) containing $10–15\times10^4$ cells. Saturating antibody concentrations were determined in previous experiments.

In three different experiments peripheral blood lymphocytes were isolated from venous

TABLE I *$CD4^+$, $CD8^+$, $CD4^-CD8^-$, and γδ T cells in the duodenum and the peripheral blood of controls and patients with HIV infection*

	Controls (n=23)			*HIV (n=8)*			*AIDS (n=26)*		
	Peripheral blood	*Duodenum*	r_s*	*Peripheral blood*	*Duodenum*	r_s*	*Peripheral blood*	*Duodenum*	r_s*
CD4/CD3 (%)	52 (41–63)†	36 (23–57)‡	−0·13	24 (22–35)‡	3 (1–6)§‖	0·14	7·5 (2–16)‡§	1 (1–2)¶‖	0·02
CD8/CD3 (%)	40 (33–50)	60 (44–75)‡	0·09	75·5 (63–78)‡	85·5 (80–94)§‖	0·02	85 (78–90)‡§	89·5 (84–92)¶‖	0·12
$CD4^-CD8^-$/CD3 (%)	6 (3–11)	4 (1–12)	0·42	4 (2–5)	5·5 (3–16)	0·73	6·5 (4–10)	10 (7–15)¶‖	0·21
CD4/CD8	1·3 (0·8–2)	0·6 (0·3–1·3)‡	−0·11	0·32 (0·28–0·56)‡	0·04 (0·01–0·08)§‖	0·05	0·09 (0·02–0·2)‡§	0·01 (0·01–0·02)¶‖	−0·17
γδ TCR/CD3 (%)	4 (3–9)	8 (4–16)‡	0·24	4 (3–11)	8 (4–10)§	0·15	6 (5–9)	12 (6–21)¶‖	0·44

*Spearman rank correlation coefficient, no significant correlation; †median (25th percentile–75th percentile); ‡$p<0·05$ compared with peripheral blood T cells of controls; §$p<0·05$ compared with peripheral blood T cells of HIV infected patients; ¶$p<0·05$ compared with peripheral blood cells of AIDS patients; ‖$p<0·05$ compared with duodenal T cells of controls.

blood of HIV infected patients by using the Ficoll centrifugation method or the complete procedure described for the isolation of intestinal lymphocytes. Flow cytometric analysis of the two differently prepared peripheral blood lymphocytes showed no significant differences for the expression of the surface antigens studies (data not shown).

Flow cytometric analysis

At least 5000 cells were analysed by flow cytometry (FACScan, Becton Dickinson, Heidelberg) and LYSIS II program (Becton Dickinson). Spectral overlap between the three fluorochromes used was eliminated by electronic compensation. Lymphocyte populations were gated by forward-sideward scatter light and by gating for $CD3^+$ cells. Gates for analysis were set to exclude more than 98% of cells stained with conjugated isotype matched control antibodies. Results are given as percentages of positive cells per $CD3^+$ T cells because the absolute number of positive cells per μl cannot be calculated in duodenal biopsy specimens.

Statistical analysis

Results were not normally distributed and therefore described as medians and range between 25th and 75th percentile. The non-parametric Spearman rank correlation coefficient was calculated to determine the correlation between the two compartments studied. The non-parametric two tailed Mann-Whitney U test for unpaired data and the two tailed Wilcoxon rank sum test for paired data were used to evaluate comparative statistical significance. p Values less than 0·05 were considered as significant.

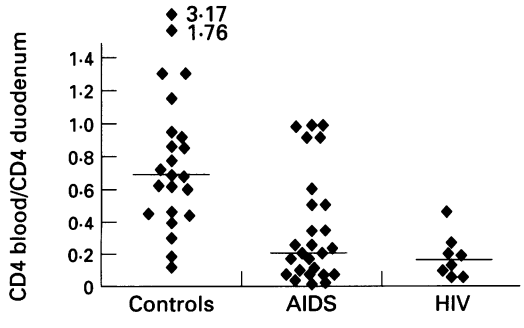

Figure 2: Ratio of duodenal to circulating $CD4^+$ T cells in controls, HIV infected patients, and AIDS patients. T cells were analysed as described in Fig 1. The ratio of duodenal to circulating $CD4^+$ T cells was decreased in AIDS and HIV infected patients compared with controls showing a preferential loss of $CD4^+$ T cells in the duodenal mucosa in HIV infection.

Results

Simultaneously isolated duodenal and circulating T cells of 23 controls, eight asymptomatic HIV infected patients, and 26 AIDS patients were studied by flow cytometry (Fig 1). The proportion of CD4 T cells in the peripheral blood decreased from 52% in controls to 24% in asymptomatic HIV infected patients ($p<0·002$ *v* controls) and to 7·5% in AIDS patients ($p<0·0005$ *v* controls; $p<0·01$ *v* HIV); correspondingly the proportion of CD8 T cells increased from 40% in controls to 75% in asymptomatic HIV infected patients ($p<0·002$ *v* controls) and to 89·5% in AIDS patients ($p<0·0005$ *v* controls; $p<0·01$ *v* HIV). The CD4/CD8 ratio decreased from 1·3 in controls to 0·32 in asymptomatic HIV infected patients ($p<0·002$) and to 0·09 in AIDS patients ($p<0·0005$ *v* controls; $p<0·01$ *v* HIV). The proportion of $CD4^-CD8^-$ and γδ T cells in the peripheral blood was not different between the three groups (Table I).

In the duodenum the proportion of CD4 T cells decreased from 36% in controls to 3% in asymptomatic HIV infected patients ($p<0·002$ *v* controls) and to 1% in AIDS patients ($p<0·0005$ *v* controls; $p>0·05$ *v* HIV; Table I). Correspondingly the proportion of CD8 T cells in the duodenum increased from 60% in controls to 85·5% in HIV infected patients ($p<0·002$ *v* controls) and to 89·5% in AIDS patients ($p<0·0005$ *v* controls; $p>0·05$ *v* HIV). The CD4/CD8 ratio in the duodenum decreased from 0·6 in controls to 0·04 in asymptomatic HIV infected patients ($p<0·002$) and to 0·01 in AIDS patients ($p<0·0005$ *v* controls; $p>0·05$ *v* HIV). The proportion of $CD4^-CD8^-$ T cells in the duodenal mucosa was not different between controls (4%) and HIV infected patients (5·5%), but increased in AIDS patients (10%) compared with controls ($p<0·025$). The percentage of γδ T cells was not different in the duodenum of the three groups (Table I).

Compared with the median proportions in controls (100%), the median proportion of $CD4^+$ T cells in the blood and duodenum was reduced to 46% and 8%, respectively, in asymptomatic HIV infected patients and to 14% and 3%, respectively, in AIDS patients. Thus the decrease in CD4 T cells in HIV

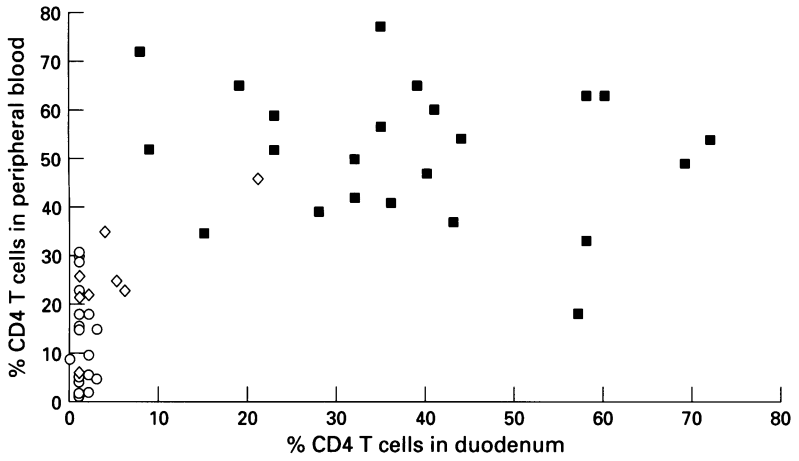

Figure 3: Correlation between the proportion of CD4+ T cells in circulating and duodenal T cells of asymptomatic HIV infected patients (open diamonds), AIDS patients (open circles), and controls (closed squares). T cells were isolated simultaneously from peripheral blood and duodenal biopsy specimens and stained with fluoresceinated antibodies to CD4, CD8, and CD3. The proportion of CD3+ T cells expressing CD4 was determined by three colour flow cytometry. No significant correlation was found between the percentage of CD4+ T cells in the peripheral blood and in the duodenum neither in controls nor in HIV infected patients.

infection is much more pronounced in the duodenum than in the peripheral blood, especially at early stages of the disease. Accordingly the ratio of duodenal to circulating $CD4^+$ T cells was significantly reduced to 0·2 (0–1) in AIDS patients ($p<0·001$) and even to 0·1 (0·04–0·5) in asymptomatic HIV infected patients ($p<0·002$) compared with 0·72 (0·44–0·95) in controls (Fig 2).

No correlation was seen in the proportion of CD4 T cells between the duodenum and the peripheral blood of controls, HIV or AIDS patients (Fig 3), or in any other T cell subset investigated between the two compartments (Table I).

The intestinal T cell subsets investigated were not different between patients with and without diarrhoea or with and without secondary intestinal infection. There were two patients with massive diarrhoea, however, who had comparatively high percentages of CD4 T cells in the peripheral blood (15% and 23%) and low proportions of CD4 in the duodenum (1% each); one patient had intestinal microsporidiosis and one patient had duodenal cytomegalovirus infection (Table II).

Discussion

The striking result of our study was that the proportion of $CD4^+$ T cells in HIV infection is about five times lower in the duodenal mucosa than in the peripheral blood. The duodenal lymphocytes studied consisted of a mixed population of intraepithelial and lamina propria lymphocytes. Normal human lamina propria T cells have a CD4/CD8 ratio similar to circulating T cells, whereas intraepithelial T cells are predominantly $CD8^+$ or $CD4^-CD8^-$.[23] Therefore the reduced percentage of $CD4^+$ cells in the duodenal T cells to about two thirds of the percentage in circulating T cells of controls is expected in the mixed population studied. The reduction in duodenal compared with circulating $CD4^+$ T cells in HIV infection, however, clearly exceeds this expected decrease, which is also evident from the reduced ratio of duodenal to circulating $CD4^+$ T cells in HIV infected patients compared with controls. The proportion of intraepithelial T cells seems to be similar in the duodenal T cells of HIV infected patients and controls as shown by the similar percentage of γδ T cells that are preferentially located in the mucosal epithelium.[23] Therefore the decreased proportion of duodenal $CD4^+$ T cells in HIV infected patients is not due to an increase in intraepithelial T cells; nor is it explained by a preferential loss of $CD4^+$ T cells in cell isolation from the duodenum as peripheral blood lymphocytes of HIV infected patients isolated by conventional density gradient centrifugation and by exactly the same isolation procedure used for duodenal lymphocytes showed no difference in $CD4^+$ T cells. Thus our findings show a profound loss of duodenal $CD4^+$ T cells in HIV infection that clearly exceeds the well known loss of $CD4^+$ T cells in the peripheral blood. Most of our patients had only minor gastrointestinal symptoms and immunological parameters were not different between patients with or without diarrhoea; although asymptomatic patients have not been investigated so far, it is therefore rather unlikely that the preferential depletion of duodenal CD4 T cells in HIV infection is restricted to patients with gastrointestinal symptoms.

Previous studies using immunohistology have shown a decreased expression of CD4 in the lamina propria of HIV infected patients compared with controls, however, this decline was highly variable with CD4/CD8 ratios of 0·1–1·1 in HIV infected patients versus 0·8–2·3 in controls.[14–18] We and others have shown recently, however, that the vast majority of $CD4^+$ lamina propria cells in the duodenum of HIV infected patients are in fact $CD4^+$ macrophages.[19 24] In addition, staining of fixed sections could also detect intracellular antigens and in vitro studies showed that CD4 expression of HIV infected T cells may be lost on the cell surface but maintained intracellularly.[25] The increase of duodenal $CD4^-CD8^-$ T cells in AIDS patients found in our study could result from such a loss of cell surface CD4, as only the surface expression of CD4 on $CD3^+$ T cells was analysed by flow cytometry.

The precise mechanism by which HIV infection leads to the $CD4^+$ T cell depletion in vivo is unclear, but it is generally thought that the amount of virus present correlates with the extent of CD4 depletion.[26 27] HIV infected cells, however, seem to be not equally

TABLE II *CD4 T cells in peripheral blood and duodenum in patients with diarrhoea or secondary intestinal infection*

	%CD4 T cells			
Patient	*Peripheral blood*	*Duodenum*	*Intestinal infection*	*Diarrhoea*
1	23	1	Cytomegalovirus	Yes
2	18	1	*Giardia lamblia*	No
3	15	1	Microsporidiosis	Yes
4	10	2	None	Yes
5	6	2	*Mycobacterium avium* complex	Yes
6	4	1	Microsporidiosis	Yes
7	2	2	*Giardia lamblia*	No
8	2	1	None	Yes

distributed throughout the immune system, but were 10-times more frequent in lymph nodes and adenoids compared with the peripheral blood,[6] and Kotler *et al* reported a 200 to 1000-fold higher HIV p24 content per wet weight in intestinal biopsy specimens compared with serum.[5] The state of differentiation of intestinal T cells that are tissue specific memory cells[13] could explain such high amounts of HIV in the intestine as memory T cells were found to be preferentially infected with HIV in the circulation.[11] In addition, the replication of HIV is probably promoted in intestinal T cells, which are more activated than peripheral blood T cells.[22] The excessive loss of $CD4^+$ T cells in the duodenum in comparison with the reduction of $CD4^+$ T cells in the peripheral blood may thus result from a higher virus load and increased viral replication in the intestinal immune system.

Numerous studies have shown a progressive decline of circulating $CD4^+$ T cells in HIV infection and the monitoring of these cells is widely used to assess and predict disease progression.[2 28] Our study clearly shows, however, that T cell subsets in the peripheral blood and the duodenal mucosa do not correlate in controls nor in HIV infected patients. This lack of correlation between circulating T cells and T cells from a lymphoid compartment actually involved in HIV disease implies that the patient's peripheral blood CD4 T cell count may be an inappropriate basis of the decision to start antiviral treatment or primary prophylaxis of opportunistic infections. Our finding of symptomatic duodenal infection with cytomegalovirus and microsporidia in two patients with comparatively high percentages of circulating CD4 T cells but nearly absent duodenal CD4 T cells supports this possibility. Largely preserved circulating $CD4^+$ T cells clearly do not permit a correct assessment of intestinal immunity as shown by the profound depletion of $CD4^+$ T cells in the duodenum in early HIV infection. Furthermore, gastrointestinal dysfunction in the absence of secondary intestinal pathogens and at early stages of HIV infection might also result from the loss of $CD4^+$ T cells in the duodenum as activated $CD4^+$ lamina propria T cells play an important part in the maintenance of the normal structure and function of the intestinal mucosa.[1 29]

In summary, we have shown that T cell subsets in the peripheral blood and the duodenal mucosa do not correlate, but $CD4^+$ T cell depletion in HIV infection is more pronounced in the duodenum than in the blood especially at early stages of HIV infection. Like HIV burden immunological abnormalities are distinct between lymphoid compartments, and profound immunodeficiency with opportunistic infection may occur in the intestine although circulating T cells are largely preserved. Thus peripheral blood CD4 T cell counts may be misleading in assessment and prediction of HIV disease and in therapeutic decisions.

The studies were supported by grants FKZ III-008-91 and 01 KI 9468 from the Bundesminister für Forschung und Technologie. We are indebted to Marion Lemke, Sylvia Münchow, Brigitta Rieger, and Gudrun Bogusch for expert technical assistance, to Ursula Littke for data documentation, and to Regina Trawinski for secretarial assistance. We thank Dr Warren Strober, Division of Intramural Research, NIAID, NIH, Bethesda, MD, USA, for critical review of the manuscript.

1 Ullrich R, Zeitz M, Riecken EO. Enteric immunologic abnormalities in HIV infection. *Semin Liver Dis* 1992; **12:** 167–73.
2 Fahey JL, Taylor JMG, Detels R, Hofmann B, Melmed R, Nishanian P, *et al.* The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *N Engl J Med* 1990; **322:** 166–72.
3 Bass HZ, Nishanian P, Hardy WD, Mitsuyasu RT, Esmail E, Cumberland W, *et al.* Immune changes in HIV-1 infection: significant correlations and differences in serum markers and lymphoid phenotypic antigens. *Clin Immunol Immunopathol* 1992; **64:** 63–70.
4 McCune JM. HIV-1: the infective process in vivo. *Cell* 1991; **64:** 351–63.
5 Kotler DP, Reka S, Borcich A, Cronin WJ. Detection, localisation and quantitation of HIV-associated antigens in the intestinal biopsies from patients with HIV. *Am J Pathol* 1991; **139:** 823–30.
6 Pantaleo G, Graziosi C, Butini L, Pizzo PA, Schnittman SM, Kotler DP, *et al.* Lymphoid organs function as major reservoirs for human immunodeficiency virus. *Proc Natl Acad Sci USA* 1991; **88:** 9838–42.
7 Embretson J, Zupanic M, Ribas JL, Burke A, Racz P, Tenner-Racz K, *et al.* Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* 1993; **362:** 359–62.
8 Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, *et al.* HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 1993; **362:** 355–8.
9 Nabel G, Baltimore D. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* 1987; **326:** 711–3.
10 Van Noesel CJ, Gruters RA, Terpstra FG, Schellekens PT, van LR, Miedema F. Functional and phenotypic evidence for a selective loss of memory T cells in asymptomatic human immunodeficiency virus-infected men. *J Clin Invest* 1990; **86:** 293–9.
11 Schnittman SM, Lane HC, Greenhouse J, Justement JS, Baseler M, Fauci AS. Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. *Proc Natl Acad Sci USA* 1990; **87:** 6058–62.
12 Zeitz M, Greene WC, Peffer NJ, James SP. Lymphocytes isolated from the intestinal lamina propria of normal nonhuman primates have increased expression of genes associated with T cell activation. *Gastroenterology* 1988; **94:** 647–55.
13 Schieferdecker HL, Ullrich R, Hirseland H, Zeitz M. T cell differentiation antigens on lymphocytes in the human intestinal lamina propria. *J Immunol* 1992; **149:** 2816–22.
14 Rodgers VD, Fassett R, Kagnoff MF. Abnormalities in intestinal mucosal T cells in homosexual populations including those with the lymphadenopathy syndrome and acquired immunodeficiency syndrome. *Gastroenterology* 1986; **90:** 552–8.
15 Budhraja M, Levendoglu H, Kocka F, Mangkornkanok M, Sherer R. Duodenal mucosal T cell subpopulation and bacterial cultures in acquired immune deficiency syndrome. *Am J Gastroenterol* 1987; **82:** 427–31.
16 Ellakany S, Whiteside TL, Schade RR, vanThiel DH. Analysis of intestinal lymphocyte subpopulations in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *Am J Clin Pathol* 1987; **87:** 356–64.
17 Ullrich R, Zeitz M, Heise W, L'age M, Ziegler K, Bergs C, *et al.* Mucosal atrophy is associated with loss of activated T cells in the duodenal mucosa of human immunodeficiency virus (HIV)-infected patients. *Digestion* 1990; **46** (suppl 2): 302–7.
18 Jarry A, Cortez A, René E, Muzeau F, Brousse N. Infected cells and immune cells in the gastrointestinal tracts of AIDS patients. An immunohistochemical study of 127 cases. *Histopathology* 1990; **16:** 133–40.
19 Lim SG, Condez A, Lee CA, Johnson MA, Elia C, Poulter LW. Loss of mucosal CD4 lymphocytes is an early feature of HIV infection. *Clin Exp Immunol* 1993; **92:** 448–54.
20 Centers for Disease Control. Classification system for human T-lymphotropic virus type III/lymphadenopathy-associated virus infections. *MMWR* 1986; **35:** 334–9.
21 Leads from the MMWR (Morbidity and Mortality Weekly Report), Supplement 15. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *JAMA* 1987; **258:** 1143–54.
22 Zeitz M, Quinn TC, Graeff AS, James SP. Mucosal T cells provide helper function but do not proliferate when stimulated by specific antigen in Lymphogranuloma venereum proctitis in nonhuman primates. *Gastroenterology* 1988; **94:** 353–66.
23 Ullrich R, Schieferdecker HL, Ziegler K, Riecken EO, Zeitz M. γ/δ T cells in the human intestine express surface markers of activation and are preferentially located in the epithelium. *Cell Immunol* 1990; **128:** 619–27.
24 Ullrich R, Schneider T, Jahn H, Bergs C, Schmidt W, Zeitz M. Preserved CD4 expression in the duodenal lamina propria of HIV-infected patients is due to macrophages.

IXth International Conference on AIDS. Berlin, 7–11 June, 1993: 439.
25 Schneider T, Hildebrandt P, Rokos K, Schubert U, Rönspeck W, Grund C, *et al.* Expression of nef, vpu, CA and CD4 during the infection of lymphoid and monocytic cell lines with HIV-1. *Arch Virol* 1992; **125:** 161–76.
26 Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N Engl J Med* 1989; **321:** 1621–5.
27 Baltimore D, Feinberg MB. HIV revealed – toward a natural history of the infection. *N Engl J Med* 1989; **321:** 1673–5.
28 Burcham J, Marmor M, Dubin N, Tindall B, Cooper DA, Berry G, *et al.* CD4% is the best predictor of development of AIDS in a cohort of HIV-infected homosexual men. *AIDS* 1991; **5:** 365–72.
29 MacDonald TT, Spencer J. Evidence that activated mucosal T cells play a role in the pathogenesis of enteropathy in human small intestine. *J Exp Med* 1988; **167:** 1341–9.